Brief Definitive Report

ACQUISITION OF CELL SURFACE IgD AFTER IN VITRO CULTURE OF NEOPLASTIC B CELLS FROM THE MURINE TUMOR BCL_1*

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The differentiation of bone marrow-derived lymphocytes in mice is characterized by the sequential appearance of different surface markers. IgM is the first isotype to appear, and IgM^+ cells subsequently acquire IgD. The acquisition of IgD appears to be antigen and T cell independent (1). The roles of these two surface isotypes in the triggering of B cells is the subject of intensive study. When mature B cells bearing both isotypes are triggered by mitogens, IgD is lost and the cells differentiate into IgM secreting plasma cells (1–3).

Recent evidence indicates that several nonsecreting B cell tumors from humans (4, 5) and mice (6) have the capacity to secrete IgM under the influence of mitogens (5), T cells (4), or after fusion to myeloma cells (7). One of the murine tumors, BCL_1, has been used in the present studies to investigate the pathway of B cell differentiation that is characterized by the acquisition of IgD. The uncultured tumor cells bear large quantities of surface IgM and trace amounts of IgD (8–10). Thus, both the phenotypic characteristics (8) and, in addition, the functional properties (9, 10) of BCL_1 cells suggest that they are analogous to immature B cells. In the present studies we have demonstrated that cultivation of BCL_1 cells results in markedly increased expression of surface IgD in the absence of IgM secretion.

Materials and Methods

Mice. BALB/c mice were obtained from Cumberland View Farms, Clinton, Tenn. The BCL_1 tumor was maintained in vivo by intravenous passage of 10^6 spleen cells obtained from a tumor-bearing mouse.

Culture Conditions. Peripheral blood and spleen cells were obtained from mice that had carried the tumor for 8–12 wk and were prepared and cultured as previously described (11)[1] without the addition of either 2-mercaptoethanol or lipopolysaccharide (LPS). Greater than 90% of the cell populations employed are tumor cells as judged by morphology and expression of idiotype (9, 12). Cells were incubated in 250-ml culture flasks (Falcon Labware, Div. of Becton, Dickinson & Co., Oxnard, Calif.) at 2×10^6/ml (30–50 ml/flask), in RPMI-1640 with 10% fetal calf serum (FCS) (Grand Island Biological Co., Grand Island, N. Y.). Under these conditions, BCL_1 cells do not secrete IgM.[1]

Immunofluorescence. Indirect immunofluorescence analysis of tumor cells on the fluorescence-activated cell sorter (FACS III, Becton, Dickinson & Co., Rutherford, N. J.) was performed as

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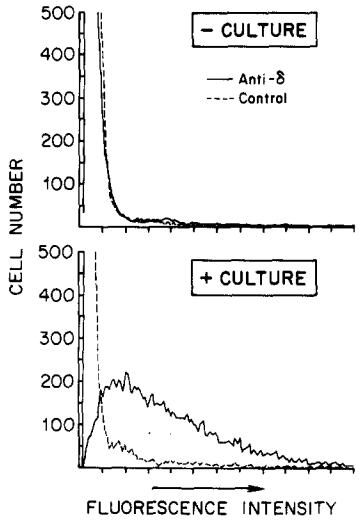

FIG. 1. Immunofluorescence analysis of BCL_1 cells stained with anti-δ. Blood cells from tumor-bearing animals were cultured at 2×10^6/ml for 4 d and, along with uncultured blood cells, were stained with hybridoma anti-δ or the control mouse IgG2A (RPC-5) and F1-RAMγ $F(ab')_2$. Cells were analyzed on the FACS, and the results were plotted as cell number vs. fluorescence intensity.

previously described (9) with either a hybridoma anti-δ (H 10.4.22) or a control IgG2A myeloma protein (RPC-5) (Litton Bionetics, Kensington, Md.), followed by incubation with fluorescein-conjugated $F(ab')_2$ rabbit anti-mouse-γ.

Cell Surface Iodination. Surface molecules were labeled with ^{125}I-Na by lactoperoxidase-catalyzed iodination, and the immunoglobulins were precipitated from the cell lysate and analyzed as previously described (12).

Radioimmunoassay for Surface Ig. The antibodies used for this assay were affinity-purified rabbit anti-mouse-μ and goat anti-mouse-δ. Preparation and iodination of the rabbit anti-μ and control Ig is detailed elsewhere.[1] Affinity-purified goat anti-mouse-δ was prepared in goats by repeated injections of 500 μg of TEPC-1017 (an IgD_κ myeloma protein [F. Finkelman, S. Kessler, F. Mushinski, and M. Potter. Manuscript in preparation.]) in adjuvant. After adsorption with an IgM_κ myeloma (TEPC-183) and BALB/c serum, the serum was affinity purified on TEPC-1033 Sepharose (Pharmacia Fine Chemicals, Div. of Pharmacia, Inc., Piscataway, N. J.) (IgD_κ) (F. Finkelman, S. Kessler, F. Mushinski, and M. Potter. Manuscript in preparation.). Tumor cells or normal spleen cells were incubated for 30 min at 4°C with 200 μl of ^{125}I-labeled antibody in 5% FCS-phosphate-buffered saline (PBS) (with 10 mM azide) followed by three washes with PBS-azide. Cells were transferred to a fresh tube and counted directly in a gamma counter. For each point, four concentrations of ^{125}I-antibody were tested, and the average counts per minute bound per 10^5 cpm input was calculated. Under these conditions, binding of ^{125}I-anti-μ and ^{125}I-anti-δ to BALB/c spleen cells was linear with respect to concentration of antibody and number of cells assayed. Binding of radioiodinated normal Ig to normal or tumor cells was always <200 cpm/10^5 input cpm.

Results

Immunofluorescence Analysis. Prior studies of BCL_1 tumor cells with both immunofluorescence and biochemical techniques demonstrated that these cells bear large quantities of surface IgM but only trace amounts of IgD (8–10). Thus, Fig. 1 shows that freshly prepared BCL_1 cells stained minimally with anti-δ. In contrast, 86% of BCL_1 cells cultured for 4 d without added mitogens stained brightly with hybridoma anti-δ but not with a control myeloma protein of the same subclass (RPC-5). The

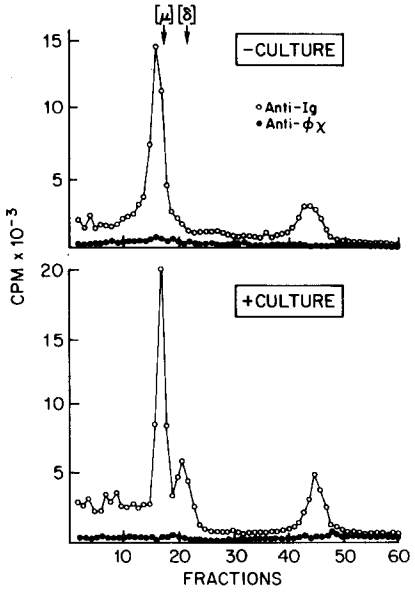

FIG. 2. Immunoprecipitation of lysates of cells radioiodinated before and after culture. BCL_1 cells, cultured for 6 d at 2×10^6/ml, were washed and iodinated; fresh BCL_1 blood cells were iodinated at the same time. The lysates were precipitated with RAMIg or control rabbit antisera (anti-φX 174) and *Staphylococcus aureus*. Complexes were eluted and electrophoresed under reducing conditions on 7.5% SDS gels.

cultured cells that stained with anti-δ were very heterogeneous with respect to fluorescence intensity, which suggests a variable density of surface IgD. This finding indicates that the vast majority of BCL_1 tumor cells differentiate in vitro and acquire the surface immunoglobulin phenotype of a more mature B cell.

Biochemical Analysis of Surface Ig. To obtain biochemical evidence that cultured BCL_1 cells acquire IgD, cells were iodinated, either before or after culture, and the lysates were analyzed by immunoprecipitation and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Immunoprecipitation with rabbit anti-mouse Ig (RAMIg) of lysates from uncultured cells demonstrated substantial quantities of μ-chain but little or no δ-chain (Fig. 2). Lysates from radioiodinated cultured cells, however, contained readily detectable IgD as determined by the presence of radioactive δ-chain on SDS-PAGE. Estimation of the areas under the μ- and δ-peaks in several experiments showed at least a three- to fourfold decrease in the μ:δ-ratio of cultured cells. Analogous results with regard to δ-chain were obtained with affinity-purified goat anti-δ (data not shown).

Radioimmunoassay for Surface Ig. To further quantify the relative amounts of IgM and IgD on cultured BCL_1 cells, cells were treated with radioiodinated, affinity-purified antibodies directed against μ- or δ-chains. As shown in Table I, BCL_1 cells bound substantially more ^{125}I-anti-μ than normal spleen cells (assuming 40% of normal spleen cells and 100% of BCL_1 cells are IgM positive) (9). In the experiment shown, binding of ^{125}I-anti-μ was unchanged on cells cultured for 6 d; in other experiments, increased binding of ^{125}I-anti-μ (up to a twofold increase) was often observed. In contrast, binding of ^{125}I-anti-δ to uncultured BCL_1 cells, although

TABLE I

Radioimmunoassay for Cell Surface IgM and IgD on BCL_1 Cells

Cell source	Days in culture*	Bound to cells		μ:δ‖
		^{125}I-anti-μ‡	^{125}I-anti-δ§	
		cpm		
BALB/c spleen¶	0	598	2,582	0.2
BCL_1 spleen**	0	2,764	933	3.0
BCL_1 spleen**	6	2,529	3,144	0.8
BCL_1 blood**	0	2,467	855	2.9
BCL_1 blood**	6	2,540	2,603	1.0

* Cells were cultured at 2×10^6/ml.
‡ 1×10^5 cpm input (2×10^6 cpm/μg).
§ 2×10^5 cpm input (2×10^6 cpm/μg).
‖ cpm anti-μ bound:cpm anti-δ bound.
¶ 5×10^6 cells/tube were assayed.
** 2×10^6 cells/tube were assayed.

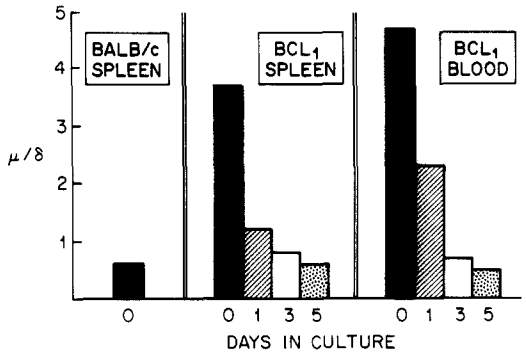

FIG. 3. Kinetics of appearance of IgD during in vitro culture. BCL_1 blood and spleen cells were cultured at 2×10^6/ml for 1, 3, or 5 d. Binding of ^{125}I-anti-μ and ^{125}I-anti-δ was performed as described in Materials and Methods; 2×10^6 BCL_1 cells or 5×10^6 BALB/c spleen cells were assayed. μ:δ-ratios were calculated on the basis of counts bound per 1×10^5 cpm added for anti-μ, and 2×10^5 cpm added for anti-δ.

reproducibly higher than the control, was very low compared with binding to normal cells. After cell culture there was a dramatic increase in ^{125}I-anti-δ binding to BCL_1 cells (Table I) but no increase in binding of the control Ig (data not shown). The increased binding of ^{125}I-anti-δ after culture is reflected in the decreased binding ratio of anti-μ:anti-δ (μ:δ). The kinetics of appearance of IgD are shown in Fig. 3 and indicate that increased levels of surface IgD could be detected as early as 1 d of culture and levels continued to increase until they plateaued at 3–5 d. It should be emphasized that because the binding assays were performed under nonsaturating conditions, the μ:δ-ratios calculated here are relative ones and do not reflect absolute amounts of surface Ig.

Discussion

The present studies indicate that culture of BCL_1 tumor cells can result in a markedly increased expression of cell surface IgD. The IgD was measured by three techniques: immunofluorescence using the FACS, immunoprecipitation of radioiodinated surface immunoglobulin and analysis by SDS-PAGE, and a radioimmunoassay with heavy chain-specific antibodies. The results of the latter assay suggest that the

density of IgD on uncultured BCL_1 cells is ~1/10th of that found on normal adult spleen cells. After 3–5 d of culture, the density of IgD increased three- to fourfold. Earlier studies have shown a loss of surface IgD during differentiation of B cells into plasma cells (1–3) but to our knowledge, this is the first demonstration of in vitro acquisition of surface IgD.

A significant development in B cell immunology was the demonstration by Fu et al. (4, 5) and Kishimoto (13) that neoplastic human B lymphocytes could be stimulated in vitro to differentiate into Ig-secreting plasma cells. Similar observations have been made with the BCL_1 tumor, i.e., cultivation of BCL_1 cells with LPS stimulates them to secrete IgM (11) bearing the same idiotype as the surface Ig of the unstimulated cells (12). The present findings extend the concept that tumor cells can differentiate. Thus, cultivation of BCL_1 cells in the absence of LPS results in differentiation along a different pathway, i.e., acquisition of an Ig phenotype characteristic of a more mature B lymphocyte. It is unclear whether this is a spontaneous maturation event related to removal of host suppressive influences or caused by trace amounts of stimulatory factors (growth factors or mitogens) in the FCS. Regardless, the BCL_1 cells may provide a useful model for analysis of the events involved in expression of IgD and the factors that determine which pathway of differentiation is chosen by B cells.

Summary

Murine BCL_1 tumor cells bear large amounts of surface IgM and trace amounts of surface IgD. In the present studies we have shown that cultivation of these cells, in the absence of lipopolysaccharide, results in the acquisition of IgD by virtually all the cells. These results suggest that BCL_1 cells can differentiate in vitro into more mature B cells and offer an attractive model for analyzing the factors controlling appearance of IgD on a monoclonal cell line.

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